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INFLUENCE OF CARBOHYDRATE LEVELS AND ROOT-SURFACE MICROFLORAS ON PHYMATOTRICHUM ROOT ROT IN COTTON AND MAIZE PLANTS [1]

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INTRODUCTION

During its fruiting period cotton (*Gossypium* spp.) is highly susceptible to attack by the root rot fungus *Phymatotrichum omnivorum* (Shear) Dugg. The fact that *P. omnivorum* attacks some 2,000 species of plants (*23*, *26*) [3] indicates that the parasitic activity of this fungus is not highly specialized. Under sterile conditions it makes a vigorous vegetative growth on diverse synthetic media (*3*, *12*, *24*, *27*) and forms sclerotia when grown on synthetic media as well as on various plant materials (*7*, *17*). The organism has been observed to die out in decaying organic material, but food exhaustion is not primarily responsible for this result (*20*); with the removal of competitive effects of other microbes by sterilization, the fungus grows well after reinoculation, regardless of the amount of decomposition that the material has previously undergone.

The actual penetration of *Phymatotrichum omnivorum* into the cells of a host plant is preceded by enzymatic and other chemical action that kills the protoplasts in advance of cell-wall destruction (*27*, *p. 59*; *32*,*33*). The foregoing findings, taken in conjunction with the fact that the organism does not become systemic in its host, point to the conclusion that it not only does not require but that it never utilizes living materials as such. In other words, this parasite appears to function as a saprophyte abundantly equipped with extracellular enzymes.

The conclusion that death of the cell precedes penetration points to an additional probability, namely that the action of *Phymatotrichum omnivorum* on and in roots releases nutrients favorable to the growth of other micro-organisms that advance with it. The existence of *P. omnivorum* within attacked tissues would thus be highly competitive, and its growth and survival would depend largely upon the relative suitability of the tissue substrate for this fungus and for competitive or antibiotic saprophytes.

The investigation reported here deals with (1) the relation between carbohydrate levels and susceptibility of cotton to phymatotrichum

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[3] Italic numbers in parentheses refer to Literature Cited, p. 160.

root rot, (2) the effects of carbohydrates in the roots of the cotton plant on the root-surface microflora, and (3), by means of sterile cultures, the protection afforded by the root microflora against the invasion and growth of the fungus on the roots of young cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) plants.

REVIEW OF LITERATURE

It is well known that some of the obligate parasites grow most vigorously in normal, well-nourished, host plants. Literature showing poor growth of rust and mildew in leaves low in carbohydrates has been reviewed by Trelease and Trelease (*31*). Somewhat less is known about the relation of the root-infecting fungi to the composition of the host plant. Leach (*18*) has reported that, while *Armillaria mellea* can develop only on roots high in carbohydrates, *Rhizoctonia bataticola* and *Botryodiplodia theobromae* are capable of developing in roots of low carbohydrate content. Infections by *A. mellea* were reduced in newly planted land by ring-barking the trunks of forest trees prior to clearing for tea gardens. According to Garrett (*13, p. 44*), Gadd has shown that failure of tea plants to recover after too frequent plucking and pruning appeared invariably to be associated with the absence of starch reserves in the roots and that this condition actually preceded infection by *Botryodiplodia*. Roots infected by *Poria, Rosellinia,* and *Ustulina* showed normal starch content.

Several investigators have given consideration to relations between susceptibility to phymatotrichum root rot and carbohydrate levels in roots and in nutrient media. Blank (*2*) ascribed the resistance of young cotton plants to their low carbohydrate content. The addition of carbohydrates to agar and soil substrates supporting germinating cotton seeds has been said by Watkins (*32*) and Blank (*2*) to increase infection. Watkins and Watkins (*33, 34*) reported ready infection of seedlings of cotton, corn, and retama when germinated on agar rich in carbohydrates. Reduction in the viability of *Phymatotrichum omnivorum* on cotton roots following girdling has been considered by Ezekiel (*11*) in connection with an associated reduction in the carbohydrate concentration of the roots. Ergle (*9*), Ergle at al. (*10*), and Talley [4] have found the carbohydrate concentrations in cotton plants to vary with age.

The general subject of disease protection by root microflora has been considered by various investigators. Lochhead (*19*) believed it possible that resistance to certain root diseases may be connected with the selective action of root excretions upon the saprophytic microflora, thus favoring types that may be more antagonistic to pathogenic micro-organisms in some cases and in others less. The presence of hydrocyanic acid in solutions in which resistant varieties of flax had been grown led Timonin (*30*) to suggest that root excretions of cyanogenetic glucosides or of hydrocyanic acid from resistant varieties either affect pathogenic fungi directly or else stimulate other micro-organisms, which in turn may influence or control the activity of pathogenic fungi.

That the number of micro-organisms associated with plant roots changes with the aging of the plants has been demonstrated by

[4] TALLEY, P. J. [Unpublished data.]

Starkey (*22*) and Mitchell, Hooton, and Clark (*20*). The last-named investigators noted also that in mechanically injured cotton plants the number of micro-organisms associated with root surfaces increased greatly during the late summer or early fall. In a continuation of this work Clark (*5*) found a change in types of saprophytic fungi as well as increased difficulty of recovery of *Phymatotrichum omnivorum* after injury. In a paper on the take-all disease of cereals Garrett (*14*) pointed out that the interest in microbiological antagonism has not led to new control measures of practical value. However, the value of organic manures in stimulating the soil microflora, in helping to alter the microbial equilibrium, and in hastening the elimination of undesirable fungi from soil, presumably through microbial antagonisms or competition, has been emphasized (*6, 16*); and changes in root-surface microfloras with changes in the soil microflora in response to the addition of soil amendments have been discussed (*6*). An appreciation of the significance of interactions between organisms may frequently be essential to the interpretation of experimental results and thereby serve to orient root-disease investigations.

MATERIAL AND METHODS

INOCULATIONS

For a study of the differences or changes in the susceptibility of plants to injury by *Phymatotrichum omnivorum*, an ideal inoculum is one that introduces the organism into each substrate uniformly as to quantity, activity, virulence, and amount of reserve food material. In the past, sclerotia, which are variable in size and activity, and pieces of infected root or other plant material, which are variable in nutrient reserve and in age of fungus, have been used as inoculum in a variety of ways. These past procedures did not seem to meet the requirements of the present investigation, and a new method was accordingly devised. By the procedure here described, a like number of milo (*Sorghum vulgare* Pers.) seeds infected with the fungus were introduced into the soil substrates at measured depths. The underlying purpose of this procedure was to make the number of days from inoculation to permanent wilting a reliable measure of the rate of upward growth of the fungus on the roots of the plant as well as of the ability of the fungus to invade and destroy.

The inoculum to be used was prepared by placing a layer of large milo seeds (previously autoclaved for 15 minutes with an equal weight of water) on well-established nutrient-agar plates of *Phymatotrichum omnivorum*. After an incubation period of 6 to 10 days at 28° C., the fungus was always well established in the seed, and there still remained within the seed a reserve of food for the subsequent development of mycelium.

In inoculating cotton plants growing in potted soils, three infected seeds were separated from the plate and dropped to the bottom of each of two holes made in the soil with a smooth, blunt rod. These holes were 8 to 16 cm. in depth, according to the age of the plants, and were placed 3 cm. on either side of the taproot. They were filled in with loose soil, and water was added to the soil surface. The sterile cultures were inoculated in a transfer room by pushing, with a sterile glass rod, three seeds through the glass watering tubes into the substrate. Importance was attached to uniform procedures

within each experiment, but the several experiments cannot be closely compared because of temperature and other differences affecting fungus activity.

CHEMICAL ANALYSES

The plant samples were preserved and the sugars extracted with hot 80-percent alcohol. Lead acetate and sodium oxalate were used in clarifying and deleading the solutions. The analyses for total sugars were made on solutions that had been inverted with hydrochloric acid for 24 hours at room temperature. Starch was determined by treating for 1 hour, boiling, and re-treating the residues, after sugar extraction, with diastase [5] at 55° C. until the material gave no color with iodine solution. The residues from the starch hydrolysis were then refluxed with 2-percent hydrochloric acid for 2.5 hours, and the sugars obtained were reported as hemicelluloses. When starch was not determined, the residues from the alcoholic extraction were hydrolyzed with 2-percent hydrochloric acid, and the results were expressed as polysaccharides, the latter being equivalent to the sum of starch and hemicelluloses as determined separately in the other procedure.

The sugar analyses on plants grown in the greenhouses were conducted according to the method of Wildman and Hansen (*35*), except that potassium permanganate oxidizing solution and orthophenanthroline indicator were used in the titration. For the field samples, the cuprous titration method of Schaffer and Hartmann was employed.

STERILE CULTURES

Those who have attempted to grow plants on sterile substrates are familiar with the many difficulties involved. In the present undertaking it was necessary to select a substrate other than coarse sand because cotton plants growing in nonsterile sand succumb slowly and irregularly to root rot after inoculation.

The wilting of plants provides a good criterion of root destruction, and it was therefore regarded as desirable, if not essential, that the tops of the plants should be freely exposed to the greenhouse atmosphere; this required provision for frequent additions of sterile water. The sterile-culture procedure finally developed represents a composite of points of technique derived from the literature and new adaptations regarded as advantageous to this particular study.

A sand-bentonite mixture was extensively employed as the substrate for the plants, but in the early phases of the study soil was also used as a substrate. A good growth of plants was obtained after repeatedly washing soils in the autoclave, drying, and then twice dry-autoclaving the culture jars after they were filled, but the percentage of sterile cultures by this procedure was always low. The sand-bentonite mixture consisted of 80 percent of 20- to 40-mesh quartz sand, 15 percent of pulverized quartz sand, and 5 percent of bentonite. To reduce alkalinity, the ground quartz sand was treated with acid and washed. Iron was supplied by mixing 0.3 percent of magnetite with the substrate material, and Hoagland's nutrient solution was added to the substrate before it was autoclaved. The hydrogen-ion concentration of the substrate was usually within the pH range 7.0 to 7.6.

Two-quart mason jars were used for vessels in all sterile-culture tests (see fig. 5). Each of the jars was fitted with an 8-mm. glass

[5] Undiluted diastase with low sugar content furnished by courtesy of Parke, Davis & Co

tube through which the plants were watered, inoculated, and sampled for sterility. The lower end of the tube extended about 6 cm. beneath the surface of the substrate through a glass sleeve (2 cm. in diameter by 6 cm. long) filled with coarse sand that served both for anchorage and for the distribution of water. The central portion of the watering tube was wrapped with cotton and tied inside the neck of the culture jar. The upper end of the tube, which extended well above the culture jar, was covered with a glass cap on a cotton shoulder. This cap was removed only in a transfer room. Cotton shoulders were tied around the outside of the neck of the culture jars and fitted with 600-ml. beakers with kraft-paper aprons. These beakers remained in place during autoclaving and after planting until the plant stems extended well above the tops of the jars.

Maize seeds and acid-delinted Stoneville 2–B cotton seeds were surface-sterilized in 6-gm. lots in 50-ml. Erlenmeyer flasks by covering them with 0.2-percent mercuric chloride in 50-percent alcohol and shaking vigorously for 2 minutes. This solution was then decanted off, and the seeds were washed three times with sterile water. After being washed, the seeds were covered a fourth time with sterile water and allowed to swell for 18 hours. The water was then decanted, and the seeds were transferred to potato-dextrose agar in Petri dishes for 40 hours. Uniformly germinated seeds from clean plates were selected for planting, and those with either shorter or longer hypocotyls were left on the plates for a further check on sterility. With this method, contaminations in the agar plates were rare and none of the germinated seeds from clean plates developed organisms when transferred to beef-extract peptone solution. The germinated sterile seeds were placed in depressions in the surface of the cultures and covered with the substrate. The control cultures were always treated and planted in the same manner as the sterile cultures, except that a small quantity of fresh soil was mixed in to reinoculate them after autoclaving and the beakers and cotton and paper covers were omitted to avoid seedling infections (damping-off and molds).

When the tops of the plants were well above the shoulders of the jars, the beakers were removed in a transfer room and sterile cotton pads were pressed firmly into place in the necks of the jars around the plant stems. The cotton shoulders that supported the beakers were removed at this time, and two sheets of light paper were then tied over the cotton pads. A second thin layer of cotton was placed above the paper, and over this a second paper covering. All these coverings were carefully fitted around the plant stems. The cultures were then weighed, to provide a basis for subsequent watering, and placed in the greenhouse in constant-temperature baths set at 28° C.

Much difficulty was encountered at first in maintaining sterile substrates with the tops of the plants freely exposed, but in the later experiments half or more of the cultures usually remained sterile for sufficiently long periods. It was customary to take samples of the substrate for plating at the time of planting and inoculation and subsequently at the times of watering. The latter samples were collected through the watering tubes by means of sterile glass tubes fitted with glass plungers. The increase in number of contaminated cultures with time may have represented contaminants introduced either by watering and sampling operations or by the infiltration of organisms along the plant stems.

CARBOHYDRATE LEVELS AND ROOT ROT

GREENHOUSE EXPERIMENTS

COTTON PLANTS OF FRUITING AGE

In two explanatory greenhouse experiments, plants of Rogers Acala cotton (*Gossypium hirsutum*) were grown in stone jars filled with 3 parts of Houston Black clay and 1 part each of Lufkin fine

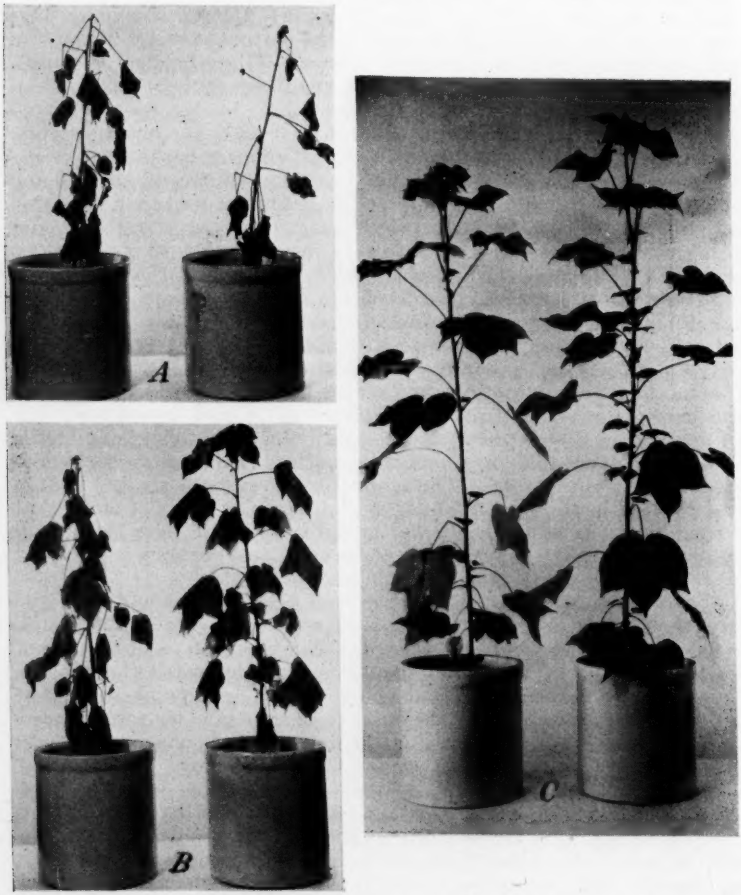

FIGURE 1.—Cotton plants of the first greenhouse experiment 13 days after inoculation: *A*, Fruited plants with half of each leaf cut away; *B*, fruited control plants; *C*, plants with all branches and bolls removed.

sandy loam, river sand, and well-decomposed manure. The pots were weighed several times each week as a watering guide to avoid any important differences in the moisture content of the soil.

In experiment 1 there were three treatments (fig. 1), each with one plant in each of seven 2-gallon jars, as follows: A, Fruited plants

with half of each leaf cut away; B, fruited control plants; C, plants with all branches and bolls removed. The results of experiment 1 are shown in the following tabulation:

Treatment: [1]	*Average period (days) from inoculation to wilting* [2]
A. Half leaf	12.7
B. Control	13.4
C. Branches and bolls removed	19.4

[1] Planted Sept. 20; treatments started Dec. 17; inoculated Jan. 18 at depth of 15 cm.
[2] Difference required for significance at 1-percent level, 5.8 days.

In experiment 2 there were two treatments, each with one plant in each of eighteen 1-gallon jars. Five days after inoculation, three jars from each treatment were selected at random for analyses of the root bark. The treatments were as follows: A, Fruited plants with two-thirds of each leaf cut away (starting 10 days before inoculation); B, plants with all branches and bolls removed (starting 10 days before inoculation) and given supplementary illumination of about 400 foot-candles (measured at the elevation of the upper leaves) for 4 hours in the early morning and 4 hours in the late afternoon. The results of experiment 2 are shown in table 1.

TABLE 1.—*Effect of treatments on carbohydrate concentrations in the root bark and on resistance of cotton plants to phymatotrichum root rot; experiment 2*

[Planted Oct. 3; treatments started Jan. 31; inoculated Feb. 11 at depth of 12 cm.]

Treatment	Root-bark carbohydrates [1]			Average time from inoculation to wilting
	Total sugars	Polysaccharides	Total	
	Percent	*Percent*	*Percent*	*Days*
A. Plants with one-third of leaf area	1.48	3.75	5.23	17.1
B. Plants with main-stalk leaves only	2.41	11.31	13.72	([2])

[1] These samples were taken 5 days after inoculation and at that time there were few lesions on the roots. Percentages expressed on fresh-weight basis.
[2] All plants were alive on the 50th day.

The results of experiments 1 and 2 were alike in indicating that high-carbohydrate cotton plants are more resistant to root rot than low-carbohydrate plants. The differences in the carbohydrate levels in the root bark were probably more marked in experiment 2 than in experiment 1 because of greater increases in carbohydrates after sampling due to extra illumination of the B plants in experiment 2 and to the smaller quantity of nitrogen supplied after inoculation.

All the high-carbohydrate plants of experiment 2 were alive 50 days after inoculation, but when the roots were removed from the soil it was found that all had been actively infected (fig. 2) at some earlier stage of the test. The progress of the disease under treatment B had evidently been slow from the start and eventually was checked, as indicated by the marked overgrowth of the bark above the rotted portions of the roots. This overgrowth was not unlike that which appears above stem girdles, and it is reasonable to believe that the final checking of the disease was associated with carbohydrate concentrations in excess of the 13.72 percent found 5 days after inoculation; a further accumulation of carbohydrates logically would follow the destruction of the lower root system.

A third experiment was projected for the twofold purpose (1) of providing four carbohydrate levels and thereby aiding a differentiation between any effects on root rot resistance that might be associated

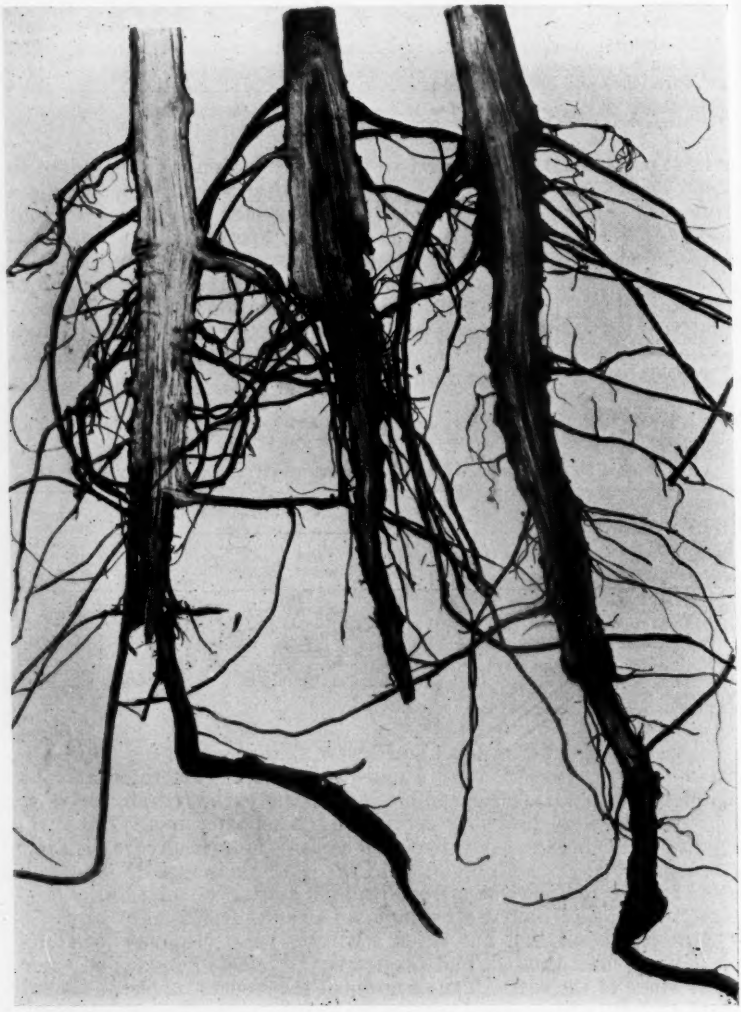

FIGURE 2.—Roots from the high-carbohydrate cotton plants of the second greenhouse experiment (table 1), showing checking of the disease and overgrowth of root bark.

with the mutilations or nitrogen accumulation, as distinct from the carbohydrate effect, and (2) of providing material for microbial assays and thereby the determination of possible relations between carbohydrate levels and equilibria of the root-surface saprophytes.

The four treatments were as follows: A, Fruited plants, normal nitrogen; B, fruited plants, low nitrogen; C, mutilated plants (all branches, bolls, and terminal buds removed), normal nitrogen; D, mutilated plants (all branches, bolls, and terminal buds removed), low nitrogen.

The seeds (Stoneville 2–B) were planted in 3-gallon jars containing 1 part Houston Black clay, 2 of Lufkin fine sandy loam, and 1 of sand. An equal amount of complete nutrient solution was added to all jars shortly after the seeds were planted, and during their early growth the plants were green and vigorous. By the time the first bolls were formed, however, growth was being checked by nitrogen deficiency. When there were from 2 to 4 bolls per plant, the pots

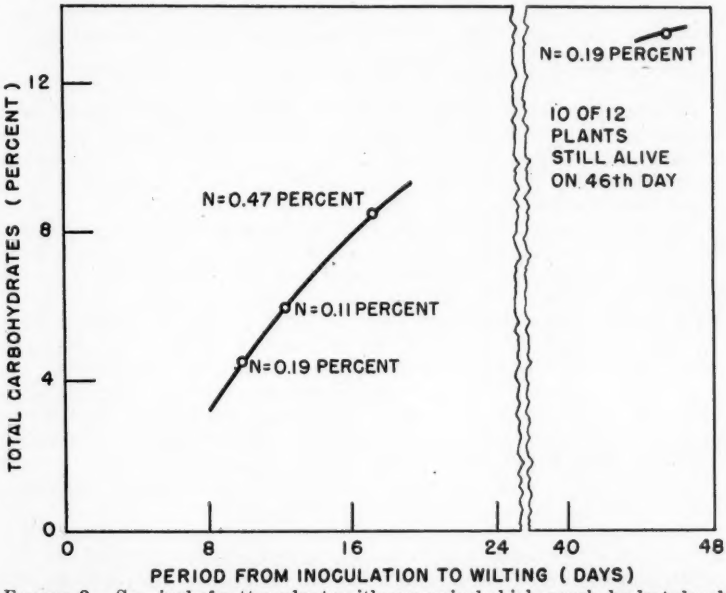

FIGURE 3.—Survival of cotton plants with successively higher carbohydrate levels in their root bark. Nitrogen concentrations in the root bark are shown numerically at each point.

were randomized into 4 groups of 17 each (except group D, 18 pots) and the differential treatments were started by adding a complete nutrient solution to groups A and C (normal-nitrogen plants) and only potassium and phosphate to groups B and D (low-nitrogen plants). All branches, bolls, and terminal buds were removed at this time from groups C and D (mutilated plants).

Sixteen days after the differential treatments were started and just prior to inoculation, six representative plants were selected from each treatment to provide duplicate samples of three plants each for the chemical analyses and microbial assays. The roots of these plants were collected 5 days after the plants to be tested for susceptibility had been inoculated. One-half of each root-bark sample, taken longitudinally, was used for chemical analyses (table 2 and fig. 3) and the other half for microbial assays (see table 7). The root samples were collected at 7 a. m.

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TABLE 2.—*Effect of nitrogen supply and mutilations on carbohydrate and nitrogen concentrations in the root bark and on resistance of cotton plants to phymatotrichum root rot, experiment 3*

[Planted Oct. 3; differential treatments started Jan. 6; inoculated Jan. 22; sampled Jan. 27]

Treatment	Root-bark composition [1]				Average period from inoculation to wilting
	Total sugars	Polysaccharides	Total carbohydrates	Total nitrogen	
	Percent	*Percent*	*Percent*	*Percent*	*Days*
A. Fruited; normal N	1.24	3.21	4.45	0.19	10.0
B. Fruited; low N	1.96	3.88	5.84	.11	12.4
C. Mutilated; normal N	1.77	6.57	8.34	.47	17.4
D. Mutilated; low N	2.23	10.82	13.05	.19	[2]

[1] Percentages expressed on fresh-weight basis.
[2] 2 of the 12 plants died on the 21st and 22d day, respectively. The others remained alive.

The relation found in experiment 3 between carbohydrate accumulation in the root bark and the rapidity of kill by the fungus was notably direct, the successively higher carbohydrate concentrations being paralleled by corresponding delays in plant death. Of the 12 high-carbohydrate plants (treatment D) 10 remained alive.

The 10 high-carbohydrate plants that remained alive were subjected to some further experimentation. Forty-six days after inoculation, nitrogen was added to 5 of these pots and the plants were allowed to produce new branches. Of the 5 plants 4 died of root rot within the next month (2 died in 8 days and 2 in 26 days) and 1 remained alive. A root section of the surviving plant is shown in figure 4. Nitrogen was added to the remaining 5 pots 122 days after inoculation, and from that date on the plants were allowed to develop new branches. None of these plants died, and 3 that were not pulled out for root examinations set and matured a crop of cotton on their new branches. This fact shows that the root rot fungus, given time, will eventually disappear from diseased cotton roots if the carbohydrate concentrations are maintained at a sufficiently high level.

The results of the experiment (table 2 and fig. 3) indicate that differences in nitrogen supply and in growth habit affected the susceptibility of these cotton plants to root rot only as they affected the carbohydrate levels in the roots. The most susceptible plants (group A) and the most resistant ones (group D) had equal total nitrogen concentrations, whereas the plants of intermediate susceptibility (groups B and C) had the lowest and highest nitrogen levels, 0.11 and 0.47 percent, respectively. Moreover, there was no apparent break in the continuity of the carbohydrate root rot curve (fig. 3) in passing from nonmutilated to mutilated plants. In other words, a smooth curve was obtained through the first three treatments irrespective of the means (nitrogen or mutilation) adopted for varying the carbohydrate levels.

Under other conditions Streets (*23*), by using heavy applications of nitrogen, was able to invigorate trees infected with *Phymatotrichum omnivorum*, and Adams et al. (*1*) found reductions in root rot on some, but not all, soils when nitrogen was applied with a drill a few inches from the cotton seed at time of planting. The mechanism of the protective action of nitrogen sometimes observed in the field is not known.

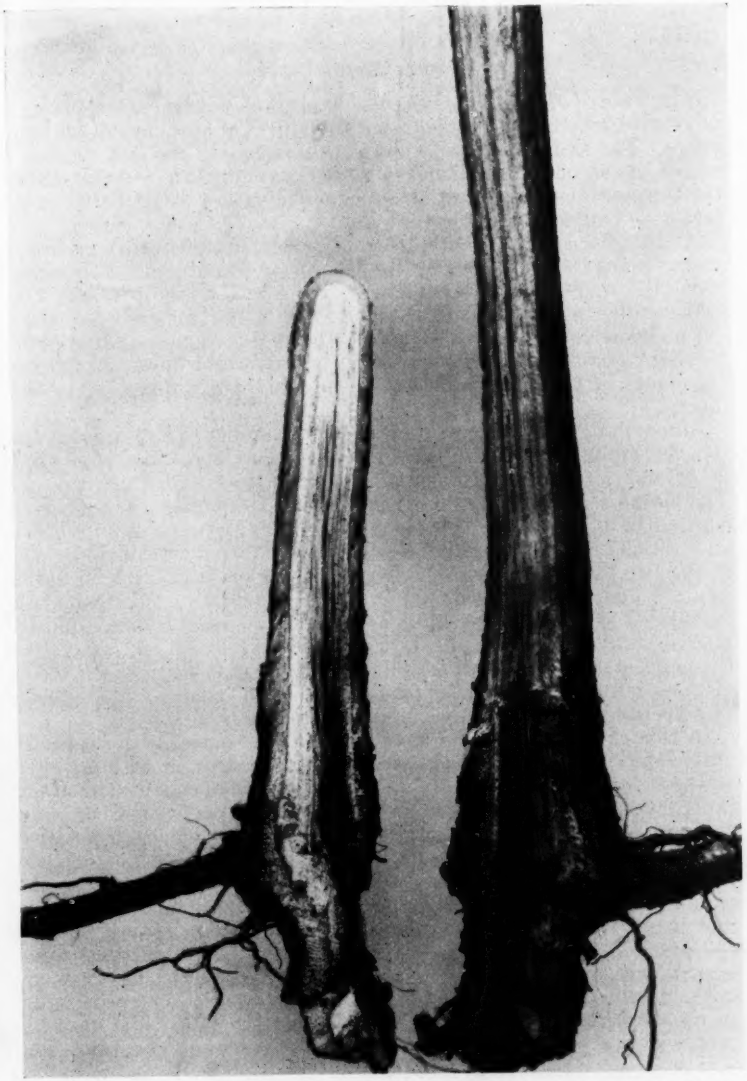

FIGURE 4.—The two halves of a root of a high-carbohydrate cotton plant of experiment 3 that recovered from root rot. There was no evidence that living *Phymatotrichum omnivorum* was present when this plant was removed from the soil 113 days after inoculation and 67 days after nitrogen was added and branches were allowed to develop. Note destruction of taproot by root rot, callusing of the root stump, and development of two strong lateral roots that supported the plant.

The effects of the treatments on the microbial assays of the root-surface saprophytes are reported in a later section of this paper (p. 153).

Young Cotton Plants

The nature of the resistance mechanism of young cotton plants to phymatotrichum root rot has been the object of speculation for many years. The experiment reported in this section is one of a number in which the carbohydrate levels of young cotton plants were altered for the purpose of determining whether any relation exists between this factor and susceptibility to root rot.

A difference in the carbohydrate levels between two groups of young plants was effected by the use of (1) Mazda lamps to supplement the midwinter illumination in the greenhouse by about 2,000 foot-candles (at the tops of the plants) over a 14-hour daily period and (2) a cheesecloth shade to reduce the normal greenhouse illumination to about one-third. Between bright and overcast days and between the hours of the day there was an unavoidable wide range in light intensity.

Stoneville 2–B cotton was planted on November 18 in 2-quart mason jars containing a mixture of Houston Black clay and Lufkin fine sandy loam and 300 ml. of Hoagland's solution was added to each jar. The plants were taken to the greenhouse on December 1 and placed in a water bath at 28° C. Supplementary illumination was supplied to all until December 20, when the differential lighting was started. One of the two plants was removed from each jar on January 1 for carbohydrate and nitrogen analyses. On the same date each culture was inoculated with *Phymatotrichum omnivorum* by placing three infected milo seeds at the bottom of a hole 5 cm. deep and 3.0 cm. away from the plant stem. The analytical data reported in table 3 are the means of three samples of five or six plants each. The analyses are reported as percentages of the fresh weight of entire plants.

In this experiment (table 3), a twofold difference in the carbohydrate concentrations of entire 44-day-old plants was without effect on the percentage of plants dying or on the time of death. In similar experiments with yet younger cotton plants, the percentages of plants dying under the two treatments were lower and, as in this experiment, there was no indication that carbohydrate levels affected the incidence of the disease. Direct comparisons between the carbohydrate concentrations found in the entire plants of this experiment and in the root bark of the fruiting-age plants of the preceding experiments are not possible, because of the difference in the plant fractions represented; but, as previously noted, carbohydrate levels tend to be low in young cotton plants. The results of the experiment are regarded as noteworthy in that not all the plants were killed and that the carbohydrate levels exerted no apparent effect upon resistance.

It has been observed by others that cotton (*32*, *33*) and corn (*34*) seedlings germinated in vitro on agar are attacked by *Phymatotrichum omnivorum*, and the use of substrates rich in carbohydrates has been reported (*2*, *32*, *33*, *34*) to increase infection. The resistance of young cotton plants has been attributed to their low carbohydrate concentrations (*2*). The data reported in table 3 fail to disclose any effect upon resistance of changes in carbohydrate levels within young cotton plants. Cotton seedlings germinated on nutrient agar and heavily

overrun by mycelium, with the formation of extensive lesions, have been found by the writers to recover and develop normally when transferred to greenhouse soils.

TABLE 3.—*Effect of carbohydrate levels on the incidence of phymatotrichum root rot in cotton plants 44 days old when inoculated*

[Percentages based on fresh weight of entire plants]

Treatment	Carbohydrates			Nitrogen	Susceptibility to root rot		
	Total sugars	Polysaccharides	Total		Plants surviving	Plants dying	Average period from inoculation to wilting
	Percent	*Percent*	*Percent*	*Percent*	*Number*	*Number*	*Days*
Shaded	0.12	1.46	1.58	0.29	4	12	12.1
Lighted	.78	2.32	3.10	.35	3	15	12.2

The possibility that the resistance of cotton seedlings might be due to an inhibiting substance resulting from photosynthetic activity was considered but was discarded on the basis of observations that followed the inoculation of agar substrates supporting sterile cotton seedlings grown in Erlemeyer flasks (1) exposed to greenhouse light (supplemented by Mazda lamps) and (2) kept in the dark. The exposed and dark-chamber flasks rested on trays in the same constant-temperature bath. The rate of destruction of the seedling tissues by the fungus was relatively slow and essentially equal regardless of whether the plants were exposed to greenhouse light or confined in the dark chamber. This fact is regarded as showing that resistance was not increased by photosynthetic activity. The supporting nutrient-agar substrates employed in the foregoing experiment were made up both with and without calcium, but the calcium was likewise without effect on the severity of the attack.

FIELD EXPERIMENTS

Relation of Root Spread to Root Rot

An important distinction exists between the development of phymatotrichum root rot in potted soils, where each plant is inoculated by some standard procedure, and the spread of the disease from natural infection centers in the field. In the field, the fungus is carried over in resting or active stages from the preceding year and its initial attack on scattered plants results from the meeting of roots and fungus somewhere in the soil mass. The enlargement of root rot spots has been described so often that it requires no elaboration here, except for one fairly obvious point. If it is granted that root rot, after attacking primary plants, spreads to surrounding plants principally by the growth of the fungus along roots and from the roots of one plant to those of neighboring plants, then the abundance, distribution, and overlapping of roots are significant factors in the spread of the disease. The following experiment is believed to illustrate the bearing of root spread upon the spread of root rot.

Three rows of cotton plants were selected in 1941 in a field that had a high root rot mortality during the previous year. When the plants were about 18 inches tall, row 2 was thinned to a spacing of

about 2 feet, which left 122 plants in this row; rows 1 and 3, containing respectively 413 and 438 plants, were not disturbed. On September 15 the percentages of dead cotton in rows 1, 2, and 3 were 68, 33, and 64, respectively. The reduced mortality of the plants in row 2 is believed to have resulted from the reduction in the overlapping and intermingling of the roots of the plants in this row. The thinning of row 2 was performed after the plants were in an advanced floral-bud stage, and the subsequent plant and root development did not compensate for the plants removed. The foregoing situation is in contrast to that existing in spacing experiments in which thinning is carried out in the seedling stage and by midsummer the entire soil space is extensively occupied by roots. In the latter type of experiment, spacing has been found to have little effect on either root rot or yield.

For several reasons it is more difficult to evaluate differences in plant resistance in field experiments than in pot experiments. In the field, values for the period from natural inoculation to wilting are not ascertainable; treatments that affect resistance may also affect spread of the roots and thereby the movement of the fungus from plant to plant. Furthermore, the reduction in population by deaths reduces the chance for a like number of new deaths during a succeeding week. In the field, root rot characteristically appears as islands of dead plants, and the chances of infection of all plants under a treatment are not the same.

The correlation between carbohydrate levels and resistance to root rot as developed in the greenhouse experiments was subjected to further test under field conditions by mutilation and cultural-practice tests conducted in 1941 near Brenham, Tex., on Houston Black clay.

Mutilation of Cotton Plants

Three plant treatments were employed in the field mutilation experiment as follows: A, Fruited plants with one-half of each leaf cut away; B, normal control plants; C, main-stalk plants (all branches and bolls removed). The plants were grown (1) on land where a high percentage of root rot had occurred during the preceding year and (2) on land of the same soil type one-quarter of a mile distant where no root rot had occurred. The latter plants provided material for the chemical analyses reported in table 4. Both of these experimental plantings were laid out in blocks (15 on the root rot land and 20 on the root-rot-free land) of 15 plants each. Within each block, 5 consecutive plants were taken for each of treatments A, B, and C, the position of these treatments being randomized within the blocks. Stoneville 2–B cotton was planted on May 6 and when about 1 foot tall was thinned to plants about 15 inches apart. The mutilation treatments were started on June 27 and repeated on the same plants once each week thereafter. The first flowers opened during the week ending July 11, and the first deaths from root rot occurred during the week ending July 18.

In the plots on root rot land, cutting away half of each leaf reduced the average number of bolls (not counting those less than 5 days old) on the longest fruiting branch from 1.43 to 0.67, shortened the average length of this branch from 27.6 to 20.9 cm., reduced the average plant height from 67 to 60 cm., and, as indicated by weight of pulled taproots in the replicate experiment (table 4), substantially reduced

root development. Reducing leaf area (table 4) had little, if any, effect on the carbohydrate levels in the root bark as determined in duplicate 20-plant samples collected about 1 hour after sunrise on two dates. The fact that the half-leaf treatment markedly retarded plant development and boll setting implies that it also substantially reduced photosynthesis, but a reduction in carbohydrates was not reflected in the early-morning samples.

TABLE 4.—*Effect of mutilation treatments upon the carbohydrate and nitrogen concentrations in root bark and upon root development in cotton plants*

[Percentages expressed on a fresh-weight basis]

Treatment	Collection date	Root-bark carbohydrates					Total nitrogen	Relative weight of pulled taproots
		Total sugars	Starch	Hemicellulose	Total	Average for both dates		
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
A. Half leaf	July 31	2.27	0.75	2.96	5.98	5.70	0.14	0.82
	Aug. 14	1.68	.63	3.10	5.41		.15	.78
B. Control	July 31	2.38	.77	2.98	6.13	5.74	.13	1.00
	Aug. 14	1.87	.65	2.83	5.35		.14	1.00
C. Main stalk	July 31	2.64	2.04	2.85	7.53	7.02	.21	1.12
	Aug. 14	2.27	1.40	2.84	6.51		.21	1.49

Limiting plant development to the main stalk (branches and bolls removed, table 4) increased the average plant height from 67 to 108 cm., resulted in enlarged and thickened leaves, and accentuated root development (compare *8*). It also substantially increased the concentration of total sugars and more than doubled the concentration of starch in the root bark.

The onset of disease was somewhat more rapid in the control plots (table 5) than in either the half-leaf or main-stalk plots, and this trend was continued to the end of the season. The average of the percentages of remaining live plants that died each week was also somewhat higher in the control plots than in the half-leaf or the main-stalk plots.

TABLE 5.—*Effect of mutilation treatments upon the occurrence of root rot in cotton plants under field conditions*

[The mutilation treatments were started June 27 and repeated once each week thereafter]

Date of count	Plants found dead under indicated treatment		
	Half leaf	Control	Main stalk
	Percent	*Percent*	*Percent*
July 18	1	1	0
July 25	3	8	3
Aug. 1	9	17	5
Aug. 8	12	21	11
Aug. 15	31	39	31
Aug. 22	48	52	36
Aug. 29	49	60	45
Sept. 5	56	68	55
Sept. 12	60	73	60
Deaths weekly, average [1]	9.3	13.3	9.4

[1] Percentage of remaining live plants dying each week.

Differences in carbohydrate levels between the half-leaf and control plants having been negligible, the only explanation of the apparent resistance of the half-leaf plants that has seemed reasonable to the writers is one that concerns the root-spread factor. All plants in each treatment of this experiment were in a single row, and it is possible that the restricted root development of the half-leaf plants retarded the spread of root rot to and among them from plants in the same or in the adjacent untreated rows.

The main-stalk plants had higher carbohydrate concentrations than the control plants, and the indication of retarded development of root rot among these plants is in keeping with the findings on the effects of high carbohydrate levels in the pot experiments. The main-stalk plants had more extensive roots than the control plants, but if these roots tended to increase the spread of root rot it seems evident that this effect was more than offset by the carbohydrate effect.

Various Cultural Practices

A cultural-practice experiment was conducted in the 2 fields that were used for the mutilation experiment. In this factorial-design experiment (4 factors each at 2 levels) the treatments were as follows: (1) 2 plants in hills 14 inches apart versus 2 plants in hills 28 inches apart, (2) plus and minus dusting for the control of boll insects, (3) plus and minus fertilization (100 pounds nitrogen per acre), and (4) plus and minus fall plowing. There were thus 16 treatment combinations. The experiment was replicated in 4 blocks, with each individual plus and minus treatment applied to a total of 32 plots on the root rot land; but on the root-rot-free land, from which the samples were drawn in duplicate for the chemical analyses, there was only a single block of the 16 treatment combinations.

Spacing, dusting, and nitrogen fertilization did not result in statistically significant differences in the percentages of plants killed by root rot, but fall plowing reduced root rot significantly. An average of 25.3 percent of the plants were killed by root rot in the fall-plowed plots, whereas 39.0 percent of the plants were killed in the plots without fall plowing. Notwithstanding the lack of significant effects upon root rot by the spacing, dusting, and nitrogen-fertilization treatments, the changes in root rot resulting from these treatments were found to be correlated with the changes in the sugar concentration of the plants as measured on the root-rot-free land. Fall plowing, which had a large effect on root rot, had no effect upon the carbohydrate concentration. The correlations between total sugars and root rot in both the fall-plowed and non-fall-plowed blocks (considered separately) were in general higher than those between total carbohydrates and root rot. The correlations found between total sugars and root rot are shown in table 6.

The results obtained from this field experiment are consistent with the carbohydrate root rot relations found in the field-mutilation and in the greenhouse experiments. The negative correlation coefficients show that the percentages of dead plants decreased as the carbohydrate concentrations increased.

TABLE 6.—*Correlation between total sugars and percentages of cotton plants dying from phymatotrichum root rot in a factorial experiment*

Dates of observation of indicated correlated items		r[1] obtained for plots—	
Total sugars	Percentage of plants killed	Fall-plowed	Not fall-plowed
July 31	As of Aug. 8	−0.46	−0.58
July 31 and Aug. 15	As of Aug. 15	−.71	−.74
Aug. 14	Between Aug. 15 and 22	−.43	−.66

[1] r required for significance, −0.50 at 5-percent level and −0.71 at 1-percent level.

ROOT-SURFACE MICROFLORAS AND CARBOHYDRATE LEVELS [6]

Although conceivably a changed root-bark composition might so affect plant resistance as to be directly responsible for the increased survival periods noted during the course of the foregoing experiments, it is also possible that changes in root-bark carbohydrate concentrations might affect the numbers or types of micro-organisms associated with root surfaces and so allow the operation of antibiotic factors. It has not been shown heretofore that differences in plant carbohydrate levels, such as those here found, produce changes in root-surface microfloras. Half portions of the root bark collected January 27 from plants in greenhouse experiment 3 were subjected to microbiologic examination.

Total numbers of bacteria were enumerated by plate-count procedure on sodium albuminate agar. Bacterial populations (expressed per gram of root-bark material on a moist-weight basis) are shown in table 7. The reduction in total bacterial numbers between the fruited cotton plants (A and B) and the plants without bolls (C and D) were highly significant.

TABLE 7.—*Bacterial numbers associated with roots of cotton plants 21 days after differential treatments (experiment 3) were established*

Treatment	Total carbohydrates	Total nitrogen	Blue-green fluorescent bacteria	Spores of aerobic *Bacillus* spp.	Total bacteria
	Percent	*Percent*	*Number* [1]	*Number* [1]	*Number* [1]
A. Fruited; normal N	4.45	0.19	166	1,924	926,680
B. Fruited; low N	5.84	.11	222	1,814	733,560
C. Mutilated; normal N	8.34	.47	204	1,389	291,595
D. Mutilated; low N	13.05	.19	901	1,472	384,485

[1] Expressed as thousands per gram of moist root-bark material; each value recorded is the average of determinations on 2 samples.

To determine whether treatment that affected carbohydrate levels resulted in differences in types of bacteria present, two subgroups of bacteria, namely, the blue-green fluorescent *Pseudomonas-Phytomonas* and the aerobic *Bacillus* spp., were enumerated. The fluorescent group, known to be relatively more prevalent on roots than in soil, was determined, by a serial dilution tube method, on a glycerol-asparagine medium (*4*). Spores of the aerobic *Bacillus* spp., relatively less prevalent on roots than in soil, were determined on nutrient beef agar after pasteurization of root-bark material in dilute aqueous

[6] By Francis E. Clark and Roland B. Mitchell.

suspensions at 85° C. for 15 minutes. Data for these subgroups are shown in table 7. In general, the number of *Bacillus* spores differed with treatments in much the same manner as did the total bacterial number. Fluorescent bacteria, on the contrary, were least numerous with the lowest carbohydrate content and most numerous with the highest. It would appear, therefore, that both the total bacterial number and the relative prevalence of at least some bacterial subgroups are influenced by the physiological status of the plant.

Some relations between total numbers of bacteria associated with roots and plant susceptibility to root-rotting parasites have been noted previously by others. Working with tobacco plants, Thom and Humfeld (*28*) noted that varieties susceptible to tobacco root rot showed higher root-surface micropopulations than did nonsusceptible varieties. Similarly, Timonin (*29*) and Lochhead (*19*) have found higher numbers of bacteria in the rhizospheres of flax and tobacco varieties susceptible to root rots than in those of rot-resistant varieties. As their results were demonstrated with uninfected plants, Lochhead believed the differences observed were due to inherent differences in physiological function, making, in the case of susceptible plants, conditions somewhat more favorable for general bacterial development. Results of the work here reported suggest that it is possible to produce, within a single variety of cotton, differences in bacterial numbers and root rot susceptibility somewhat analogous to those that exist between different varieties of flax and tobacco.

Whether the reduction in the total number of bacteria and the possible selective encouragement of particular bacterial subgroups following differential treatments are responsible for decreased root rot susceptibility is not answered; however, the fact that the microbial equilibrium is disturbed does suggest that different microbial interrelations or antibiotic phenomena were involved.

ROOT-SURFACE MICROFLORAS AND ROOT ROT RESISTANCE

COTTON AND MAIZE IN STERILE CULTURES

If the micro-organisms that populate the root surfaces of any plant provide protection against a root pathogen, that fact should be most clearly shown by growing the plant on a substrate in which the pathogen is the only micro-organism present. Besides the possibility that certain members of the saprophytic population may actually aid parasitism, the roots of plants growing in sterile cultures should be more quickly or more severely attacked than those of control plants with a normal complement of root saprophytes.

As a means of testing the idea that antibiotic activity of root-surface micro-organisms might be involved in the resistance of certain plants to phymatotrichum root rot, cotton and maize were grown on sterile sand-bentonite substrates. (See p. 140.) Young cotton plants have long been observed to be comparatively resistant to root rot under field conditions, and the immunity of corn was mentioned by Pammel (*21*) as early as 1888. Taubenhaus and Ezekiel (*25*), in a study of the resistance of monocotyledons to *Phymatotrichum omnivorum*, were unable to obtain any evidence of infection of corn interplanted with cotton that died following soil inoculations.

The results of all the experiments with the young cotton plants (Stoneville 2–B) were of the same general pattern, whether the substrates were inoculated at the time of planting or after the plants had been in the greenhouse for several weeks. The percentage of plants killed by root rot was always relatively low (ranging from 10 to 30 percent) in both sterile and control cultures. In some experiments a slight advantage seemed to lie with the control cultures and in others with the sterile cultures, but in no experiment was there substantial evidence of significant differences between the two treatments, either in the percentage of plants dying or in the number of days from inoculation to wilting. All the experiments indicated that any protection afforded by the root-surface saprophytes of young cotton

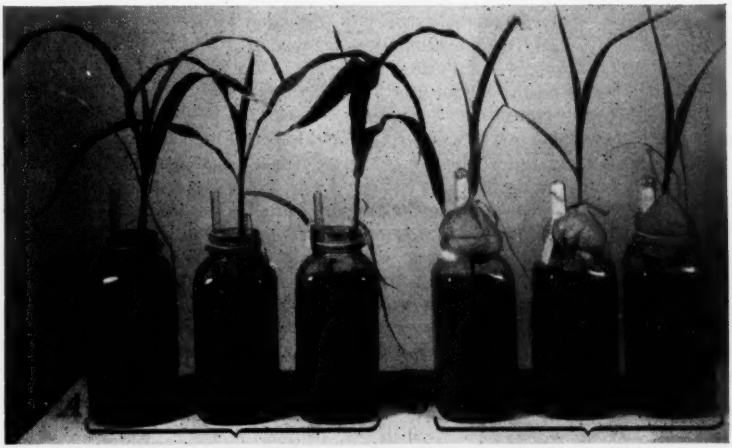

FIGURE 5.—*A*, Healthy maize plants growing in nonsterile control cultures. *B*, Dying plants in initially sterile cultures following inoculation with *Phymatotrichum omnivorum;* many infected roots were observed along the walls of the culture vessels.

plants was secondary to chemical or other resistance factors. That the culture methods, the substrate, and the viability and infectivity of the inoculum employed in the investigation were suitable for critical tests is shown by the results of the experiments with maize.

Two experiments were conducted with maize plants. An F_1 hybrid (Kansas Sunflower × Kansas Yellow Saline) was used in the first experiment and Yellow Surcropper in the second. The procedures followed in both of these tests were the same as those employed in the later and more satisfactory experiments with cotton. The results obtained from each of the two maize experiments were the same and each was conclusive. The plants in the control and sterile cultures grew equally well prior to and for a few days after inoculation with *Phymatotrichum omnivorum*. Beyond that time, however, the maize plants in all the sterile cultures declined rapidly, the leaves wilted and curled (fig. 5), and the plants died. The maize plants on the nonsterile substrates continued for the duration of the experiments to make a normal, healthy growth.

After the inoculations, in the experiments both with cotton and with maize, mycelium and strands of *Phymatotrichum omnivorum* grew out to the walls of the glass jars and developed extensively. This visible growth of the fungus, like the growth of the maize roots, appeared to be equal in the control and sterile cultures. As time progressed the roots of the maize came to be paralleled for long distances and crossed repeatedly by the fungus strands. In this respect there was no difference in the appearance of the control and sterile cultures. In the control cultures, however, maize roots in contact with the fungus remained bright and there was no evidence of any lesions. On the other hand, in the sterile cultures which had no organisms present other than *P. omnivorum*, many lesions developed and long sections of the roots turned brown and died. Furthermore, when the sterile-culture plants were removed from the jars, diseased roots were found throughout the sand mass and the lower portions of the crowns had likewise been attacked. In the nonsterile control cultures no evidence of diseased roots could be found.

The external strands of *Phymatotrichum omnivorum* were removed with sterile forceps from the surface of a number of segments of diseased roots from the sterile cultures. Some of these segments were planted directly on nutrient agar while others were surface-sterilized with 0.1-percent mercuric chloride in 50-percent alcohol and then planted. Growth was obtained on all of these plates, showing that the fungus was present within the roots as well as upon them. The results of these experiments would seem to indicate beyond any reasonable question that the maize plants were killed by the root rot fungus and that the immunity of the maize plant to phymatotrichum root rot is attributable in part, if not entirely, to protection afforded by its root-surface microflora.

Additional evidence that the resistance of seedling cotton plants to *Phymatotrichum omnivorum* may be chemical and that of maize plants primarily antibiotic was provided by other laboratory observations. When cotton seeds were germinated under sterile conditions on agar plates and the substrate was then inoculated with *P. omnivorum*, the attack remained slow and uncertain until a large mass of the fungus had developed. When maize, on the other hand, was similarly germinated, the hypocotyls were freely overrun by the developing fungus and the tissues were rapidly invaded.

The observation that cotton seedlings, after having been overrun and attacked by a mass of *Phymatotrichum omnivorum*, recover when transplanted from nutrient agar to greenhouse soils has a further bearing on the chemical interpretation of resistance. The fungus, when supported in a vigorous condition by an external substrate, produced lesions on the meristematic tissues of the cotton seedlings; but, as viewed in retrospect, it seems probable that the lesions represented only the extracellular activity of the fungus and that the seedling tissue was in itself unsuitable as a substrate for the growth of this fungus.

COMPANION PLANTS

In view of the marked differences known to exist in the number and kind of microbes populating the rhizospheres of different plant species, it seemed worth while to learn whether the resistance of cotton

to *Phymatotrichum omnivorum* might be affected by growing it in immediate proximity to other plants. For the purpose of a test, cotton was planted in the greenhouse in jars of soil in which there were also planted onions, beans, ryegrass, alfalfa, winter peas, vetch, mustard, beets, carrots, and Icicle radishes. Following the previously described procedure, *P. omnivorum* was introduced into the soils when the cotton plants were in an advanced floral-bud stage. Only with radish, which has been shown by Clark (*4*) to have an unusually large number of fluorescent bacteria associated with its roots, was there any suggestion of an increased resistance of the cotton. The effect of radishes on the resistance of cotton was subsequently tested by planting them in the same hills with cotton in short replicated rows at the Temple Substation of the Texas Agricultural Experiment Station. The growth of the radishes was satisfactory under the field conditions, but no effect was observed upon the susceptibility of the cotton plants to root rot. The possibility remains that under other conditions or by the use of other plants some measure of resistance might be gained by growing companion plants in the same hills with cotton, but the chances for success now seem somewhat remote.

DISCUSSION

In each of a series of greenhouse and field experiments with cotton plants of fruiting age, a direct correlation was found between the carbohydrate concentration of the root bark and the resistance of cotton to phymatotrichum root rot. Plants with low carbohydrate concentration usually died within the second week after inoculation, the death of plants with intermediate concentration was substantially delayed, and plants high in carbohydrates remained alive. In all experiments the high-carbohydrate plants suffered initial lesions after inoculation, but the progress of the disease up the taproot was slow and customarily stopped before it reached the uppermost lateral roots. If the plants were maintained in the high-carbohydrate condition for several months, the fungus disappeared from the roots and normal growth was then possible. The complete recovery was probably associated with additional carbohydrate accumulation consequent to the disrupted translocation and to the reduction in water supply caused by the loss of the lower roots.

One cannot conclude from the foregoing data that high carbohydrate concentrations in plant tissues are directly unfavorable to *Phymatotrichum omnivorum* without neglecting the results of extensive laboratory investigations wherein the organism has shown excellent growth in vitro on high-carbohydrate substrates. If *P. omnivorum* were the only organism present when tissues are attacked, its growth should be enhanced rather than retarded by a richer substrate. Under natural conditions, however, saprophytic organisms are also present and the growth of the root rot fungus upon and within plant tissue must in some measure always be competitive. In such systems the growth and survival of *P. omnivorum* on a tissue substrate become dependent upon the relative suitability of the substrate for the various members of the microbial population and upon the interactions between these organisms. In other words, the absence of competition with other organisms differentiates the pure-culture reactions of *P. omnivorum* from those resulting from the ecological relations that exist in natural habitats.

The assays of the root-surface microflora of the cotton plant at four carbohydrate levels showed that the physiological status of this plant has a notable effect on the saprophytic flora of its root surface. Although there were more fluorescent blue-green bacteria, there were fewer *Bacillus* spores and the total number of bacteria was smaller on cotton roots having high carbohydrate concentrations than on those having low carbohydrate concentrations. The fact that the total bacterial number was decreased suggests that the organism or organisms responsible for checking or destroying *Phymatotrichum omnivorum* may also have been unfavorable to other groups. These assays do not show that organisms in themselves were responsible for the suppression and eventual disappearance of *P. omnivorum* from the roots, but they do show that the microbial equilibria were altered with changes in carbohydrate levels, and it is reasonable to assume that without these changes in the microflora *P. omnivorum* would not have been eliminated.

The spread of *Phymatotrichum omnivorum* along the roots of cotton plants low in carbohydrates proceeded with remarkable rapidity, and there was apparently little inhibition to the destruction of the epidermal and underlying tissues. When the plants were high in carbohydrates, on the other hand, the progress of the disease within the tissue mass was slow and eventually stopped. The inhibiting factor, here presumed to be antibiotic, was not limited to the tissue mass but was also a surface effect, inasmuch as the epidermal tissues of high-carbohydrate plants above the lesions remained relatively clean whereas in low-carbohydrate plants epidermal degradation under the mycelial weft is fully evident far in advance of the more deeply rotted regions.

Although correlations and circumstantial evidence may lead to reasonable conclusions on the activity of root-surface organisms in the protection of plants against root diseases, such conclusions remain uncertain in the absence of a demonstration that immunity or resistance is lost when the plant is grown on a sterile substrate.

Young cotton plants have long been observed to be resistant to phymatotrichum root rot, and it has been shown (*20*) that their root-surface microfloral equilibria are notably different from those found on the roots of older cotton plants. Young cotton plants, however, were found to remain resistant to root rot when grown on sterile substrates, and this resistance was not altered when their carbohydrate levels were altered. Circumstantial evidence supports the conclusion that the resistance of high-carbohydrate cotton plants of fruiting age is attributable to antibiotic activity, but a final demonstration that such is the case would necessitate growing plants of this age on sterile substrates. No method of accomplishing this with the tops of the plants freely exposed is known at the present time.

The possible broad significance of the antibiotic factor in the resistance of plants to phymatotrichum root rot was indicated by experiments with maize. Maize plants growing on nonsterile substrates are highly immune to this disease. After inoculations of sterile cultures, however, lesions were rapidly formed on the roots, the leaves wilted and rolled, and the plants died. On the other hand, no lesions were observed on the roots of the maize plants grown on the nonsterile control cultures, even though many roots were paralleled by phymatotrichum strands for long distances along the walls of the

glass culture vessels. The antibiotic protection afforded the maize plant by its root-surface organisms is apparently so great that *Phymatotrichum omnivorum* is unable to exert any effect upon the epidermal tissues.

In almost any field of cotton attacked by root rot, individual plants may be observed that are apparently more resistant to disease than neighboring plants. From such plants (*26*) and from comparisons between cotton strains, species, and their hybrids (*15*) efforts have been made to select strains of high resistance. The correlation between resistance and the effects of high carbohydrates, when considered in conjunction with the effects of fruiting and of environmental conditions on carbohydrate concentrations, serves to suggest that apparent resistance may often reflect the effects of physiological status rather than genetic factors. The most productive cotton strains are logically those that most effectively translocate and utilize the products of photosynthesis in boll production. By reason of the low carbohydrate concentrations associated under moist conditions, with high fruitfulness, the productive strains, other conditions being equal, should be the most susceptible to root rot.

Field practices or conditions that increase the productiveness of the cotton plant by increasing fruitfulness and growth, i. e., carbohydrate utilization as distinguished from carbohydrate storage, would also, on the basis of the experiments here reported, increase root rot. However, root rot rarely attacks all parts of a cotton field, and the yield of plants prior to attack is often substantial. Therefore, practices and conditions that increase productiveness, even though associated with treatments that increase the number of dead plants, usually result in net gains in yield.

CONCLUSIONS AND SUMMARY

In potted soils and in the field it was found that the susceptibility of cotton plants of fruiting age to phymatotrichum root rot is related to the carbohydrate content of the root bark. Carbohydrate levels were altered by such means as partial defoliation, defruiting, branch removal, and adjustments in nitrogen supply. With increasing carbohydrate concentration resistance was increased, and plants sufficiently high in carbohydrates withstood attack.

Seedling cotton plants are highly resistant to phymatotrichum root rot, and this resistance was not altered by changes in carbohydrate levels. Likewise, the reactions of seedling cotton plants grown on sterile sand-bentonite substrates showed the resistance to be largely independent of any protection afforded by root-surface saprophytes. A mass attack of *Phymatotrichum omnivorum* produced lesions on seedlings grown on agar substrates, but it seemed doubtful that the living seedling tissues would independently support fungus growth. That the resistance factor is independent of current photosynthetic activity was shown by the similarly weak attack on seedlings growing on agar cultures in flasks kept in a dark chamber and on those exposed to the light.

The correlation between carbohydrate concentration and the resistance of cotton to root rot is believed to reflect antibiotic protection at the higher carbohydrate levels. It was found that the microbial equilibria on the surfaces of cotton roots were markedly altered as the

carbohydrate concentration within the roots was increased. The number of certain organisms tended to increase through successive carbohydrate levels whereas others decreased.

The existence of an important interaction was demonstrated between root-surface saprophytes and the parasitic activity of the root rot fungus *Phymatotrichum omnivorum* by means of experiments with maize. After inoculation with *P. omnivorum* maize plants growing on sterile sand-bentonite substrates were rapidly attacked and killed. The roots of maize plants on otherwise similar but nonsterile substrates remained healthy even though paralleled for long distances by strands of the fungus.

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LOSS OF FURFURAL-YIELDING CONSTITUTENTS FROM WHEAT STRAW ON TREATMENT WITH ALCOHOLIC AND AQUEOUS SODIUM HYDROXIDE SOLUTIONS[1]

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INTRODUCTION

The loss of furfural-yielding constituents in the course of the isolation of hemicelluloses is a matter of considerable importance when the composition of these carbohydrate complexes is to be determined. Obviously, any treatment that results in the removal or degradation of any constituent normally forming a part of the hemicellulose complex would affect the composition of the isolated hemicellulose fraction. The study reported herein was undertaken to determine the extent of loss of furfural-yielding constituents at different stages in the isolation of the hemicelluloses of wheat straw.

REVIEW OF LITERATURE

The various hemicellulose preparations described by the early investigators were contaminated with other organic substances, particularly lignin. Norris and Preece (*7*),[2] in their studies on the hemicelluloses of wheat bran, partially delignified the bran by extracting it twice with a boiling 1-percent sodium hydroxide solution in 50-percent ethanol, each extraction being carried out under a reflux condenser for 2 hours.

Norman (*5*, *6*) found that the preliminary delignification of the plant material with hot alcoholic sodium hydroxide solution as used by Norris and Preece (*7*) resulted in a loss of furfural-yielding constituents.

Buston (*3*), in preparing the hemicelluloses from cocksfoot grass, treated the plant material with a 1-percent sodium hydroxide solution in 50-percent ethanol and allowed the mixture to digest at room temperature overnight.

Phillips and Davis (*8*) and Davis and Phillips (*4*) found that neither the lignin fractions precipitated from the cold alcoholic sodium hydroxide extracts of alfalfa and bagasse nor the filtrates from the lignin fractions yielded any furfural when distilled with 12-percent hydrochloric acid.

Angell and Norris (*1*) reported that furfural-yielding constituents could not be detected in the hot alcoholic sodium hydroxide extract of hop flowers.

[1] Received for publication July 19, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 167.

Preece (*9*) investigated the loss of furfural-yielding constituents at various stages in the process of extracting the hemicelluloses from lilac wood and teak wood. In the case of lilac wood, he found that of the 12.44 percent furfural originally present in the material, a total of 10.66 percent could be accounted for in the ammonium oxalate extract, the alcoholic alkaline extract, the hemicelluloses, and the residual cellulosic material. About 14 percent of the total furfural could not be accounted for. In commenting on this loss Preece (*9*, *p. 252*) states:

> The discrepancy between the two totals [that is between 12.44 and 10.66] is surprisingly high, and it cannot be readily accounted for unless degradative changes have occurred during extraction. It is impossible that as much as 14% of the total furfuraldehyde could be represented by normal "extraction losses."

Preece (*9*) found that the treatment of unextracted teak wood sawdust with a 4-percent aqueous sodium hydroxide solution at room temperature brought about no loss in the furfural-yielding constituents, although a hot 4-percent aqueous sodium hydroxide solution caused a 3.5 percent loss of furfural. However, teak wood sawdust which had previously been extracted with ether and alcohol and then boiled with a 4-percent aqueous sodium hydroxide solution suffered a loss of 7.2 percent of furfural. Similarly Preece (*10*) found a loss of furfural-yielding constituents as a result of pretreatment of willow and mahogany woods with boiling sodium hydroxide solutions.

EXPERIMENTAL METHODS

The ground wheat straw used in the experiments was first extracted for 30 hours in a Soxhlet extractor with a 1:2 alcohol-benzene solution and then with a hot (85° C.) 0.5-percent aqueous ammonium oxalate solution until the extract no longer gave a precipitate on the addition of ethanol. The extracted straw was dried in the steam drier.

To 200 gm. of the extracted straw sufficient 2-percent sodium hydroxide solution in 50-percent ethanol was added to immerse the plant material completely. The reaction mixture was stirred manually from time to time and allowed to stand overnight. It was then filtered, the residual material was washed with 70-percent ethanol containing some acetic acid, and the washings were added to the alkaline alcoholic filtrate. This combined filtrate was neutralized with hydrochloric acid and evaporated to dryness on the steam bath. The product obtained was ground, allowed to dry overnight at room temperature, weighed, and the percentage of furfural was determined.

The wheat straw, which had been extracted with the alcoholic sodium hydroxide solution, was dried at room temperature and weighed and a 5-gm. sample was retained for the determination of furfural. To the remainder of the wheat straw, a 4-percent aqueous sodium hydroxide solution was added, in the proportion of 1 part of straw to 15 parts of the alkali solution, the mixture was stirred manually from time to time, and allowed to stand at room temperature for 20 hours. The reaction mixture was filtered, and the previously described extraction with a 4-percent aqueous sodium hydroxide solution was repeated 3 times more, making a total of 4 successive extractions with the alkali solution. The last of these alkaline extracts showed only

a trace of hemicellulose when it was diluted with 3 volumes of 95-percent ethanol. To the combined alkaline extract, 3 volumes of 95-percent ethanol were added, and after the mixture had stood overnight, the hemicelluloses were separated with the aid of the centrifuge. The hemicellulose precipitate was first washed with 70-percent ethanol, acidified with acetic acid, and then washed successively with neutral 70-percent ethanol, 95-percent ethanol, absolute ethanol, and ether. The product was dried in vacuo over sulfuric acid, weighed, and the percentage of furfural determined.

The cellulosic residue (designated as A) from the above treatment with aqueous 4-percent sodium hydroxide solution was washed with 70-percent ethanol acidified with acetic acid, then with neutral 70-percent ethanol, dried in the steam drier and weighed, and a 5-gm. sample was taken for the determination of furfural.

The filtrate and washings from the hemicellulose preparation as well as the washings from the cellulosic residue A were combined and neutralized, and the solution was evaporated to dryness on the steam bath. The residual material on distillation with 12-percent hydrochloric acid yielded no furfural.

Cellulosic residue A was digested with a 2-percent sodium hydroxide solution in 50-percent ethanol under conditions already described. It was then washed with 70-percent ethanol containing some acetic acid, and the washings were added to the alkaline alcoholic filtrate. This solution was neutralized with hydrochloric acid, evaporated to dryness on the steam bath, and the residual material was weighed. When this material was distilled with 12-percent hydrochloric acid, it yielded no furfural.

The cellulosic residue A which had been extracted with the alcoholic sodium hydroxide solution was dried at room temperature, weighed, and a 5-gm. sample was retained for the determination of furfural. The remainder of the cellulosic residue was then extracted four successive times with a 4-percent aqueous sodium hydroxide solution, and the hemicelluloses in the alkaline extract were precipitated, washed, and dried; the procedure already described in connection with the first aqueous alkaline extraction was followed.

The cellulosic residue from the preceding operation (designated as B) was washed with water, and the washings were added to the filtrate from the hemicelluloses of the second 4-percent aqueous sodium hydroxide solution. The washed cellulosic material was first dried in the steam drier, then air-dried, weighed, and a 5-gm. sample was taken for analysis. To the remainder of this material a 4-percent aqueous sodium hydroxide solution was added in the proportion of 1 part of plant material to 15 parts of alkali solution, and the mixture was boiled under a reflux condenser for 1 hour. The reaction mixture was filtered, the residual material was washed with water, and the washings were added to the hot alkaline filtrate. The washed residual material was dried in the steam drier, then air-dried, weighed, and the percentage of furfural was determined.

The hemicelluloses in the extract obtained by boiling with the 4-percent aqueous sodium hydroxide solution were precipitated by following the procedure previously described for the isolation of the hemicelluloses and the treatment of the filtrate and washings for the determination of furfural-yielding constituents. The hemicellulose

precipitates were dried in vacuo at 60° C., weighed, and the percentage of furfural was determined.

All experiments recorded in this paper were made in duplicate, and the results given in table 1 represent the mean of two values.

The furfural determinations were made by the Tollens-Kröber method (*2*). In determining the furfural balance for each step of the operation, allowance was made for the 5-gm. samples taken for analysis.

RESULTS

The results of the determinations are recorded in table 1.

TABLE 1.—*Loss of furfural-yielding constituents in the isolation of hemicelluloses of wheat straw*

[Furfural in the 200 gm. of starting material=37.64 gm.]

(1) Plant material digested at room temperature for 18 hours with 2-percent sodium hydroxide solution in 50-percent ethanol

Furfural extracted by alcoholic N_aOH		Furfural in residual material		Loss of furfural
Gram	*Percent of total*	*Grams*	*Percent of total*	*Percent of total*
0.66	1.75	34.05	90.46	7.79

(2) Residual material from (1) digested at room temperature 4 successive times with 4-percent aqueous sodium hydroxide solution, each digestion period lasting 20 hours

Furfural in hemicelluloses		Furfural in residual material		Apparent gain in furfural
Grams	*Percent of total*	*Grams*	*Percent of total*	*Percent of total*
22.83	60.65	11.43	30.37	0.56

(3) Residual material from (2) digested at room temperature for 18 hours with 2-percent sodium hydroxide solution in 50-percent ethanol

Furfural extracted by alcoholic N_aOH		Furfural in residual material		Loss of furfural
Gram	*Percent of total*	*Grams*	*Percent of total*	*Percent of total*
0	0	11.32	30.07	0.29

(4) Residual material from (3) digested at room temperature 4 successive times with 4-percent aqueous sodium hydroxide solution, each digestion period lasting 20 hours

Furfural in hemicelluloses		Furfural in residual material		Loss of furfural
Grams	*Percent of total*	*Grams*	*Percent of total*	*Percent of total*
1.45	3.85	8.65	22.98	3.24

(5) Residual material from (4) boiled under reflux condenser for 1 hour with 4-percent aqueous sodium hydroxide solution

Furfural in hemicelluloses		Furfural in residual material		Apparent gain in furfural
Grams	*Percent of total*	*Grams*	*Percent of total*	*Percent of total*
1.44	3.82	7.50	19.92	0.77

It will be observed from table 1 that digestion of wheat straw with a 2-percent solution of sodium hydroxide in 50-percent ethanol resulted in a 9.54 percent loss in the yield of furfural of which only 1.75 percent could be accounted for in the alcoholic extract. It would appear, then, that the action of the alcoholic sodium hydroxide solution brought about an actual destruction of some labile furfural-yielding constituent affording 7.79 percent of the total furfural. Moreover, it is rather significant that the greatest loss of furfural-yielding constituents occurred at this stage of the operation; the

losses from the subsequent treatments with alcoholic sodium hydroxide solution and with cold and hot 4-percent aqueous sodium hydroxide solution were much smaller. The gains in the percentages of furfural recorded in sections (2) and (5) of table 1 are rather small and may well have been due to experimental error, although the possibility that some furfural-yielding constituents may have been formed in the course of the treatment with alkali is not excluded.

SUMMARY

A study was made to determine the extent of loss of furfural-yielding constituents at different stages in the isolation of the hemicelluloses of wheat straw.

Partial delignification of the straw with a 2-percent solution of sodium hydroxide in 50-percent ethanol caused a loss of furfural-yielding constituents which amounted to 7.79 percent of the total furfural.

Wheat straw which had been partially delignified with a 2-percent solution of sodium hydroxide in 50-percent ethanol and then subjected to the successive action of cold 4-percent aqueous sodium hydroxide solution, cold 2-percent solution of sodium hydroxide in 50-percent ethanol, cold 4-percent aqueous sodium hydroxide solution, and finally boiling 4-percent aqueous sodium hydroxide solution, suffered little or no loss of furfural-yielding constituents.

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